Characterization

Materials and Equipment

- Spectrophotometer
- Micropipette
- Micropipette tips
- Centrifuge
- Mini-petri dish
- CAM
- Sterile filter paper
- LB medium

Methodology

- Inoculate 30ml of culture 1 (BL21/DH5α + J04450). Determine OD till it reaches 0.8
- Inoculate 100ml culture 2 (BL21 + Lambda Lyzosyme cassette). Perform induction and wait 4 hours.
- Centrifuge culture to obtain pellet. 13,000 RPM
- Resuspend pellet in 2 mL of cold PBS (10 mM)
- Apply polytron (13,000 rpm, 15 s, ice for 1 min, 4 cycles).
- Centrifuge culture to obtain supernatant. 2,850 RPM for 5 min. Recover proteic extract and mix.
- Dilute as stated by the following table.

Table 1. Dilutions for the characterization essay.

Culture 1	800 ul	800 ul	800 ul	800 ul	800 ul
Proteic extract	200ul	400 ul	600 ul	800 ul	1000 ul

- Incubate at 37°C for 2h

Alternative 1

- For each dilution, plate 30 ul on a mini-petri dish (CAM). incubate at 37°C/16hrs. Count UFC of each plate.

- For each dilution, measure OD at 600nm.

Alternative 2

- Cut 6 circles (1 cm diameter) from a sterile filter paper and soak them in the following volumes of dilutions of proteic extract with LB:
    - 1:100
    - 1:10,000

- Plate competent cells (same strain as the ones used for culture 1) on a petri dish (No antibiotic). Place the filter papers on the proteic extract to form an antibiogram. incubate for 14 hrs. Determine the diameters of the inhibition halos.